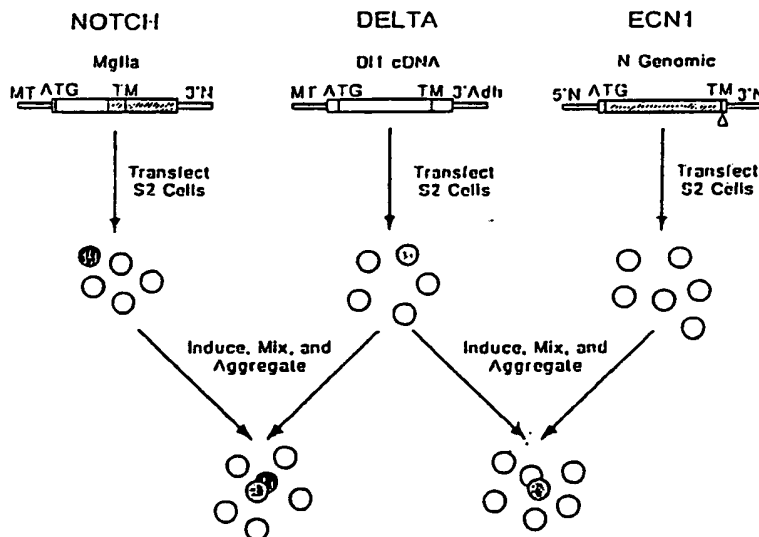




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(21) International Application Number: PCT/US92/03651 (22) International Filing Date: 1 May 1992 (01.05.92) (30) Priority data: 695,189 3 May 1991 (03.05.91) US 791,923 14 November 1991 (14.11.91) US (71) Applicants: YALE UNIVERSITY [US/US]; 216 Prospect Street, New Haven, CT 06511 (US). INDIANA UNIVERSITY FOUNDATION [US/US]; Showalter House, P.O. Box 500, Bloomington, IN 47402 (US). (72) Inventors: ARTAVANIS-TSAKONAS, Spyridon ; 192 Ridgewood Avenue, Hamden, CT 06517 (US). MUSKAVITCH, Marc, Alan, Telander ; 1308 Elliston Drive, Bloomington, IN 47401 (US). FEHON, Richard, Grant ; REBAY, Ilaria ; BLAUMUELLER, Christine, Marie ; Dept. of Biology-KBT, Yale University, 219 Prospect Street, New Haven, CT 06511 (US). SHEPARD, Scott, Brockewell ; 50, Cutler Lane, Chestnut Hill, MA 02167 (US).		(74) Agent: MISROCK, S., Leslie; Pennie & Edmonds, 1155 Avenue of the Americas, New York, NY 10036 (US). (81) Designated States: AT (European patent), AU, BE (European patent), BR, CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, KR, LU (European patent), MC (European patent), NL (European patent), NO, SE (European patent). Published With international search report.	

(54) Title: BINDING DOMAINS IN NOTCH AND DELTA PROTEINS



(57) Abstract

The present invention relates to nucleotide sequences of the human *Notch* and *Delta* genes, and amino acid sequences of their encoded proteins, as well as fragments thereof containing an antigenic determinant or which are functionally active. The invention is also directed to fragments (termed herein "adhesive fragments"), and the sequences thereof, of the proteins ("toporythmic proteins") encoded by toporythmic genes which mediate homotypic or heterotypic binding to toporythmic proteins. Toporythmic genes, as used herein, refers to the genes *Notch*, *Delta* and *Serrate*, as well as other members of the *Delta/Serrate* family which may be identified, e.g., by the methods described herein. Antibodies to human *Notch* and to adhesive fragments are additionally provided.

site at the 5' end, and proceeding in the 5' to 3' direction. Figure 21B: The DNA sequence (SEQ ID NO:21) of a portion of the human Notch insert is shown, starting near the 3' end, and proceeding in the 3' to 5' direction. The sequences shown are tentative, subject to confirmation by determination of overlapping sequences.

Figure 22. Nucleotide Sequences of Human Notch Contained in Plasmid cDNA Clone hN5k. Figure 22A: The DNA sequence (SEQ ID NO:22) of a portion of the human Notch insert is shown, starting at the EcoRI site at the 5' end, and proceeding in the 5' to 3' direction. Figure 22B: The DNA sequence (SEQ ID NO:23) of a portion of the human Notch insert is shown, starting near the 3' end, and proceeding in the 3' to 5' direction. Figure 22C: The DNA sequence (SEQ ID NO:24) of a portion of the human Notch insert is shown, starting 3' of the sequence shown in Figure 22A, and proceeding in the 5' to 3' direction. Figure 22D: The DNA sequence (SEQ ID NO:25) of a portion of the human Notch insert is shown, starting 5' of the sequence shown in Figure 22B, and proceeding in the 3' to 5' direction. The sequences shown are tentative, subject to confirmation by determination of overlapping sequences.

Figure 23. DNA (SEQ ID NO:31) and Amino Acid (SEQ ID NO:34) Sequences of Human Notch Contained in Plasmid cDNA Clone hN3k.

Figure 24. DNA (SEQ ID NO:33) and Amino Acid (SEQ ID NO:34) Sequences of Human Notch Contained in Plasmid cDNA Clone hN5k.

Figure 25. Comparison of hN5k With Other Notch Homologs. Figure 25A. Schematic representation of Drosophila Notch. Indicated are the signal sequence (signal), the 36 EGF-like repeats, the three

Notch/lin-12 repeats, the transmembrane domain (TM), the six CDC10 repeats, the OPA repeat, and the PEST (proline, glutamic acid, serine, threonine)-rich region. Figure 25B. Alignment of the deduced amino acid sequence of hN5k with sequences of other Notch homologs. Amino acids are numbered on the left side. The cdc10 and PEST-rich regions are both boxed, and individual cdc10 repeats are marked. Amino acids which are identical in three or more sequences are highlighted. The primers used to clone hN5k are indicated below the sequences from which they were designed. The nuclear localization sequence (NLS), casein kinase II (CKII), and cdc2 kinase (cdc2) sites of the putative CcN motif of the vertebrate Notch homologs are boxed. The possible bipartite nuclear targeting sequence (BNTS) and proximal phosphorylation sites of Drosophila Notch are also boxed.

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to nucleotide sequences of the human Notch and Delta genes, and amino acid sequences of their encoded proteins. The invention further relates to fragments (termed herein "adhesive fragments") of the proteins encoded by toporythmic genes which mediate homotypic or heterotypic binding to toporythmic proteins or adhesive fragments thereof. Toporythmic genes, as used herein, shall mean the genes Notch, Delta, and Serrate, as well as other members of the Delta/Serrate family which may be identified, e.g. by the methods described in Section 5.3, infra.

The nucleic acid and amino acid sequences and antibodies thereto of the invention can be used for the detection and quantitation of mRNA for human Notch and Delta and adhesive molecules, to study

expression thereof, to produce human Notch and Delta and adhesive sequences, in the study and manipulation of differentiation processes.

For clarity of disclosure, and not by way of limitation, the detailed description of the invention will be divided into the following sub-sections:

- (i) Identification of and the sequences of toporythmic protein domains that mediate binding to toporythmic protein domains;
- (ii) The cloning and sequencing of human Notch and Delta;
- (iii) Identification of additional members of the Delta/Serrate family;
- (iv) The expression of toporythmic genes;
- (v) Identification and purification of the expressed gene product; and
- (vi) Generation of antibodies to toporythmic proteins and adhesive sequences thereof.

5.1. IDENTIFICATION OF AND THE SEQUENCES OF TOPORYTHMIC PROTEIN DOMAINS THAT MEDIATE BINDING TO TOPORYTHMIC PROTEIN DOMAINS

The invention provides for toporythmic protein fragments, and analogs or derivatives thereof, which mediate homotypic or heterotypic binding (and thus are termed herein "adhesive"), and nucleic acid sequences relating to the foregoing.

In a specific embodiment, the adhesive fragment of Notch is that comprising the portion of Notch most homologous to ELR 11 and 12, i.e., amino acid numbers 447 through 527 (SEQ ID NO:1) of the Drosophila Notch sequence (see Figure 8). In another specific embodiment, the adhesive fragment of Delta mediating homotypic binding is that comprising the

- 145 -

Thr Gln Gln Val Gln Pro Gln Asn Leu Gln Met Gln Gln Gln Asn Leu
 930 935 940
 Gln Pro Ala Asn Ile Gln Gln Gln Gln Ser Leu Gln Pro Pro Pro Pro
 945 950 955 960
 Pro Pro Gln Pro His Leu Gly Val Ser Ser Ala Ala Ser Gly His Leu
 965 970 975
 Gly Arg Ser Phe Leu Ser Gly Glu Pro Ser Gln Ala Asp Val Gln Pro
 980 985 990
 Leu Gly Pro Ser Ser Leu Ala Val His Thr Ile Leu Pro Gln Glu Ser
 995 1000 1005
 Pro Ala Leu Pro Thr Ser Leu Pro Ser Ser Leu Val Pro Pro Val Thr
 1010 1015 1020
 Ala Ala Gln Phe Leu Thr Pro Pro Ser Gln His Ser Tyr Ser Ser Pro
 1025 1030 1035 1040
 Val Asp Asn Thr Pro Ser His Gln Leu Gln Val Pro Val Pro Val Met
 1045 1050 1055
 Val Met Ile Arg Ser Ser Asp Pro Ser Lys Gly Ser Ser Ile Leu Ile
 1060 1065 1070
 Glu Ala Pro Asp Ser Trp
 1075

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4268 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 2..1972

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

G GAG GTG GAT GTG TTA GAT GTG AAT GTC CGT GGC CCA GAT GGC TGC	46
Glu Val Asp Val Leu Asp Val Asn Val Arg Gly Pro Asp Gly Cys	
1 5 10 15	
ACC CCA TTG ATG TTG GCT TCT CTC CGA GGA GGC AGC TCA GAT TTG AGT	94
Thr Pro Leu Met Leu Ala Ser Leu Arg Gly Gly Ser Ser Asp Leu Ser	
20 25 30	
GAT GAA GAT GAA GAT GCA GAG GAC TCT TCT GCT AAC ATC ATC ACA GAC	142
Asp Glu Asp Glu Asp Ala Glu Asp Ser Ser Ala Asn Ile Ile Thr Asp	
35 40 45	
TTG GTC TAC CAG GGT GCC AGC CTC CAG GCC CAG ACA GAC CGG ACT GGT	190
Leu Val Tyr Gln Gly Ala Ser Leu Gln Ala Gln Thr Asp Arg Thr Gly	
50 55 60	
GAG ATG GCC CTG CAC CTT GCA GCC CGC TAC TCA CGG GCT GAT GCT GCC	238
Glu Met Ala Leu His Leu Ala Ala Arg Tyr Ser Arg Ala Asp Ala Ala	
65 70 75	
AAG CGT CTC CTG GAT GCA GGT GCA GAT GCC AAT GCC CAG GAC AAC ATG	286
Lys Arg Leu Leu Asp Ala Gly Ala Asp Ala Asn Ala Gln Asp Asn Met	

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80	85	90	95	
GGC CGC TGT CCA CTC CAT GCT GCA GTG GCA GCT GAT GCC CAA GGT GTC Gly Arg Cys Pro Leu His Ala Ala Val Ala Ala Asp Ala Gln Gly Val 100 105 110				334
TTC CAG ATT CTG ATT CGC AAC CGA GTA ACT GAT CTA GAT GCC AGG ATG Phe Gln Ile Leu Ile Arg Asn Arg Val Thr Asp Leu Asp Ala Arg Met 115 120 125				382
AAT GAT GGT ACT ACA CCC CTG ATC CTG GCT GCC CGC CTG GCT GTG GAG Asn Asp Gly Thr Thr Pro Leu Ile Leu Ala Ala Arg Leu Ala Val Glu 130 135 140				430
GGA ATG GTG GCA GAA CTG ATC AAC TGC CAA GCG GAT GTG AAT GCA GTG Gly Met Val Ala Glu Leu Ile Asn Cys Gln Ala Asp Val Asn Ala Val 145 150 155				478
GAT GAC CAT GGA AAA TCT GCT CTT CAC TGG GCA GCT GCT GTC AAT AAT Asp Asp His Gly Lys Ser Ala Leu His Trp Ala Ala Val Asn Asn 160 165 170 175				526
GTG GAG GCA ACT CTT TTG TTG TTG AAA AAT GGG GCC AAC CGA GAC ATG Val Glu Ala Thr Leu Leu Leu Lys Asn Gly Ala Asn Arg Asp Met 180 185 190				574
CAG GAC AAC AAG GAA GAG ACA CCT CTG TTT CTT GCT GCC CGG GAG GGG Gln Asp Asn Lys Glu Glu Thr Pro Phe Leu Ala Ala Arg Glu Gly 195 200 205				622
AGC TAT GAA GCA GCC AAG ATC CTG TTA GAC CAT TTT GCC AAT CGA GAC Ser Tyr Glu Ala Ala Lys Ile Leu Leu Asp His Phe Ala Asn Arg Asp 210 215 220				670
ATC ACA GAC CAT ATG GAT CGT CTT CCC CGG GAT GTG GCT CGG GAT CGC Ile Thr Asp His Met Asp Arg Leu Pro Arg Asp Val Ala Arg Asp Arg 225 230 235				718
ATG CAC CAT GAC ATT GTG CGC CTT CTG GAT GAA TAC AAT GTG ACC CCA Met His His Asp Ile Val Arg Leu Leu Asp Glu Tyr Asn Val Thr Pro 240 245 250 255				766
AGC CCT CCA GGC ACC GTG TTG ACT TCT GCT CTC TCA CCT GTC ATC TGT Ser Pro Pro Gly Thr Val Leu Thr Ser Ala Leu Ser Pro Val Ile Cys 260 265 270				814
GGG CCC AAC AGA TCT TTC CTC AGC CTG AAG CAC ACC CCA ATG GGC AAG Gly Pro Asn Arg Ser Phe Leu Ser Leu Lys His Thr Pro Met Gly Lys 275 280 285				862
AAG TCT AGA CGG CCC AGT GCC AAG AGT ACC ATG CCT ACT AGC CTC CCT Lys Ser Arg Arg Pro Ser Ala Lys Ser Thr Met Pro Thr Ser Leu Pro 290 295 300				910
AAC CTT GCC AAG GAG GCA AAG GAT GCC AAG GGT AGT AGG AGG AAG AAG Asn Leu Ala Lys Glu Ala Lys Asp Ala Lys Gly Ser Arg Arg Lys Lys 305 310 315				958
TCT CTG AGT GAG AAG GTC CAA CTG TCT GAG AGT TCA GTA ACT TTA TCC Ser Leu Ser Glu Lys Val Gln Leu Ser Glu Ser Ser Val Thr Leu Ser 320 325 330 335				1006
CCT GTT GAT TCC CTA GAA TCT CCT CAC ACG TAT GTT TCC GAC ACC ACA Pro Val Asp Ser Leu Glu Ser Pro His Thr Tyr Val Ser Asp Thr Thr 340 345 350				1054
TCC TCT CCA ATG ATT ACA TCC CCT GGG ATC TTA CAG GCC TCA CCC AAC Ser Ser Pro Met Ile Thr Ser Pro Gly Ile Leu Gln Ala Ser Pro Asn 355 360 365				1102

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CCT ATG TTG GCC ACT GCC GCC CCT CCT GCC CCA GTC CAT GCC CAG CAT	1150
Pro Met Leu Ala Thr Ala Ala Pro Pro Ala Pro Val His Ala Gln His	
370 375 380	
GCA CTA TCT TTT TCT AAC CTT CAT GAA ATG CAG CCT TTG GCA CAT GGG	1198
Ala Leu Ser Phe Ser Asn Leu His Glu Met Gln Pro Leu Ala His Gly	
385 390 395	
GCC AGC ACT GTG CTT CCC TCA GTG AGC CAG TTG CTA TCC CAC CAC CAC	1246
Ala Ser Thr Val Leu Pro Ser Val Ser Gln Leu Leu Ser His His His	
400 405 410 415	
ATT GTG TCT CCA GGC AGT GGC AGT GCT GGA AGC TTG AGT AGG CTC CAT	1294
Ile Val Ser Pro Gly Ser Gly Ser Ala Gly Ser Leu Ser Arg Leu His	
420 425 430	
CCA GTC CCA GTC CCA GCA GAT TGG ATG AAC CGC ATG GAG GTG AAT GAG	1342
Pro Val Pro Val Pro Ala Asp Trp Met Asn Arg Met Glu Val Asn Glu	
435 440 445	
ACC CAG TAC AAT GAG ATG TTT GGT ATG GTC CTG GCT CCA GCT GAG GGC	1390
Thr Gln Tyr Asn Glu Met Phe Gly Met Val Leu Ala Pro Ala Glu Gly	
450 455 460	
ACC CAT CCT GGC ATA GCT CCC CAG AGC AGG CCA CCT GAA GGG AAG CAC	1438
Thr His Pro Gly Ile Ala Pro Gln Ser Arg Pro Pro Glu Gly Lys His	
465 470 475	
ATA ACC ACC CCT CGG GAG CCC TTG CCC CCC ATT GTG ACT TTC CAG CTC	1486
Ile Thr Thr Pro Arg Glu Pro Leu Pro Pro Ile Val Thr Phe Gln Leu	
480 485 490 495	
ATC CCT AAA GGC AGT ATT GCC CAA CCA GCG GGG GCT CCC CAG CCT CAG	1534
Ile Pro Lys Gly Ser Ile Ala Gln Pro Ala Gly Ala Pro Gln Pro Gln	
500 505 510	
TCC ACC TGC CCT CCA GCT GTT GCG GGC CCC CTG CCC ACC ATG TAC CAG	1582
Ser Thr Cys Pro Pro Ala Val Ala Gly Pro Leu Pro Thr Met Tyr Gln	
515 520 525	
ATT CCA GAA ATG GCC CGT TTG CCC AGT GTG GCT TTC CCC ACT GCC ATG	1630
Ile Pro Glu Met Ala Arg Leu Pro Ser Val Ala Phe Pro Thr Ala Met	
530 535 540	
ATG CCC CAG CAG GAC GGG CAG GTA GCT CAG ACC ATT CTC CCA GCC TAT	1678
Met Pro Gln Gln Asp Gly Gln Val Ala Gln Thr Ile Leu Pro Ala Tyr	
545 550 555	
CAT CCT TTC CCA GCC TCT GTG GGC AAG TAC CCC ACA CCC CCT TCA CAG	1726
His Pro Phe Pro Ala Ser Val Gly Lys Tyr Pro Thr Pro Pro Ser Gln	
560 565 570 575	
CAC AGT TAT GCT TCC TCA AAT GCT GCT GAG CGA ACA CCC AGT CAC AGT	1774
His Ser Tyr Ala Ser Ser Asn Ala Ala Glu Arg Thr Pro Ser His Ser	
580 585 590	
GGT CAC CTC CAG GGT GAG CAT CCC TAC CTG ACA CCA TCC CCA GAG TCT	1822
Gly His Leu Gln Gly Glu His Pro Tyr Leu Thr Pro Ser Pro Glu Ser	
595 600 605	
CCT GAC CAG TGG TCA AGT TCA TCA CCC CAC TCT GCT TCT GAC TGG TCA	1870
Pro Asp Gln Trp Ser Ser Ser Ser Pro His Ser Ala Ser Asp Trp Ser	
610 615 620	
GAT GTG ACC ACC AGC CCT ACC CCT GGG GGT GCT GGA GGA GGT CAG CGG	1918
Asp Val Thr Thr Ser Pro Thr Pro Gly Gly Ala Gly Gly Gly Gln Arg	
625 630 635	
GGA CCT GGG ACA CAC ATG TCT GAG CCA CCA CAC AAC AAC ATG CAG GTT	1966
Gly Pro Gly Thr His Met Ser Glu Pro Pro His Asn Asn Met Gln Val	

640	645	650	655	
TAT GCG TGAGAGAGTC CACCTCCAGT GTAGAGACAT AACTGACTTT TGTAAATGCT				2022
Tyr Ala				
GCTGAGGAAC AAATGAAGGT CATCCGGGAG AGAAATGAAG AAATCTCTGG AGCCAGCTTC				2082
TAGAGGTAGG AAAGAGAAGA TGTTCTTATT CAGATAATGC AAGAGAAGCA ATTCGTCAGT				2142
TTCACTGGGT ATCTGCAAGG CTTATTGATT ATTCTAATCT AATAAGACAA GTTTGTGGAA				2202
ATGCAAGATG AATACAAGCC TTGGGTCCAT GTTACTCTC TTCTATTGG AGAATAAGAT				2262
GGATGCTTAT TGAAGCCCAG ACATTCTTGC AGCTTGGACT GCATTTTAAG CCCTGCAGGC				2322
TTCTGCCATA TCCATGAGAA GATTCTACAC TAGCGTCCCTG TTGGGAATTA TGCCCTGGAA				2382
TTCTGCCTGA ATTGACCTAC GCATCTCCTC CTCCTTGGAC ATTCTTTTGT CTTCAATTGG				2442
TGCTTTTGGT TTTGCACCTC TCCGTGATTG TAGCCCTACC AGCATGTTAT AGGGCAAGAC				2502
CTTTGTGCTT TTGATCATTG TGGCCCATGA AAGCAACTTT GGTCTCCTTT CCCCTCCTGT				2562
CTTCCCGGTA TCCCTTGGAG TCTCACAAAGG TTTACTTTGG TATGGTTCTC AGCACAAACC				2622
TTTCAAGTAT GTTGTCTCTT TGGAAAATGG ACATACTGTA TTGTGTTCTC CTGCATATAT				2682
CATTCTGGA GAGAGAAGGG GAGAAGAATA CTTTTCTTCA ACAAATTTTG GGGGCAGGAG				2742
ATCCCTTCAA GAGGCTGCAC CTTAATTTTT CTTGTCTGTG TGCAGGTCTT CATATAAACT				2802
TTACCAGGAA GAAGGGTGTG AGTTTGTGTG TTTTCTGTGT ATGGGCCTGG TCAGTGTAAG				2862
GTTTATCCT TGATAGTCTA GTTACTATGA CCCTCCCAC TTTTAAAG CCAGAAAAAG				2922
GTTTGAATG TTGGAATGAC CAAGAGACAA GTTAACTCGT GCAAGAGCCA GTTACCCACC				2982
CACAGGTCCC CCTACTTCCT GCCAAGCATT CCATTGACTG CCTGTATGGA ACACATTTGT				3042
CCCAGATCTG AGCATTCTAG GCCTGTTTCA CTCACTCACC CAGCATATGA AACTAGTCTT				3102
AACTGTTGAG CCTTTCCTTT CATATCCACA GAAGACACTG TCTCAAATGT TGTACCCTTG				3162
CCATTTAGGA CTGAACTTTC CTTAGCCCAA GGGACCCAGT GACAGTTGTC TTCCGTTTGT				3222
CAGATGATCA GTCTCTACTG ATTATCTTGC TGCTTAAAGG CCTGCTCACC AATCTTTCTT				3282
TCACACCGTG TGGTCCGTGT TACTGGTATA CCCAGTATGT TCTCACTGAA GACATGGACT				3342
TTATATGTTT AAGTGCAGGA ATTGGAAAGT TGGACTTGTT TTCTATGATC CAAAACAGCC				3402
CTATAAGAAG GTTGGAAAAG GAGGAACTAT ATAGCAGCCT TTGCTATTTT CTGCTACCAT				3462
TTCTTTTCCT CTGAAGCGGC CATGACATTG CTTTGGCAA CTAACGTAGA AACTCAACAG				3522
AACATTTTCC TTTCTTAGAG TCACCTTTTA GATGATAATG GACAACTATA GACTTGCTCA				3582
TTGTTGAGAC TGATTGCCCC TCACCTGAAT CCACTCTCTG TATTCATGCT CTTGGCAATT				3642
TCTTTGACTT TCTTTTAAGG GCAGAAGCAT TTTAGTTAAT TGTAAGATAA GAATAGTTTT				3702
CTTCTCTTTC TCCTTGGGCC AGTTAATAAT TGGTCCATGG CTACACTGCA ACTTCCGTCC				3762
AGTGCTGTGA TGCCCATGAC ACCTGCAAAA TAAGTTCTGC CTGGGCATTT TGTAAGATAT				3822
AACAGGTGAA TTCCCGACTC TTTTGGTTTG AATGACAGTT CTCATTCCTT CTATGGCTGC				3882
AGTATGCAT CAGTGCTTCC CACTTACCTG ATTTGTCTGT CGGTGGCCCC ATATGGAAAC				3942

CCTGCGTGTC TGTGGCATA ATAGTTTACA AATGGTTTTT TCAGTCCTAT CCAAATTTAT 4002
 TGAACCAACA AAAATAATTA CTTCTGCCCT GAGATAAGCA GATTAAGTTT GTTCATTCTC 4062
 TGCTTTATTC TCTCCATGTG GCAACATTCT GTCAGCCTCT TTCATAGTGT GCAAACATTT 4122
 TATCATTCTA AATGGTGA CTCTGCCCTT GGACCCATTT ATTATTCACA GATGGGGAGA 4182
 ACCTATCTGC ATGGACCCTC ACCATCCTCT GTGCAGCACA CACAGTGCAG GGAGCCAGTG 4242
 GCGATGGCGA TGACTTTCTT CCCCTG 4268

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 657 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Glu Val Asp Val Leu Asp Val Asn Val Arg Gly Pro Asp Gly Cys Thr
 1 5 10 15
 Pro Leu Met Leu Ala Ser Leu Arg Gly Gly Ser Ser Asp Leu Ser Asp
 20 25 30
 Glu Asp Glu Asp Ala Glu Asp Ser Ser Ala Asn Ile Ile Thr Asp Leu
 35 40 45
 Val Tyr Gln Gly Ala Ser Leu Gln Ala Gln Thr Asp Arg Thr Gly Glu
 50 55 60
 Met Ala Leu His Leu Ala Ala Arg Tyr Ser Arg Ala Asp Ala Ala Lys
 65 70 75 80
 Arg Leu Leu Asp Ala Gly Ala Asp Ala Asn Ala Gln Asp Asn Met Gly
 85 90 95
 Arg Cys Pro Leu His Ala Ala Val Ala Ala Asp Ala Gln Gly Val Phe
 100 105 110
 Gln Ile Leu Ile Arg Asn Arg Val Thr Asp Leu Asp Ala Arg Met Asn
 115 120 125
 Asp Gly Thr Thr Pro Leu Ile Leu Ala Ala Arg Leu Ala Val Glu Gly
 130 135 140
 Met Val Ala Glu Leu Ile Asn Cys Gln Ala Asp Val Asn Ala Val Asp
 145 150 155 160
 Asp His Gly Lys Ser Ala Leu His Trp Ala Ala Ala Val Asn Asn Val
 165 170 175
 Glu Ala Thr Leu Leu Leu Leu Lys Asn Gly Ala Asn Arg Asp Met Gln
 180 185 190
 Asp Asn Lys Glu Glu Thr Pro Leu Phe Leu Ala Ala Arg Glu Gly Ser
 195 200 205
 Tyr Glu Ala Ala Lys Ile Leu Leu Asp His Phe Ala Asn Arg Asp Ile
 210 215 220
 Thr Asp His Met Asp Arg Leu Pro Arg Asp Val Ala Arg Asp Arg Met
 225 230 235 240
 His His Asp Ile Val Arg Leu Leu Asp Glu Tyr Asn Val Thr Pro Ser

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245	250	255
Pro Pro Gly Thr Val Leu Thr Ser Ala Leu Ser Pro Val Ile Cys Gly 260	265	270
Pro Asn Arg Ser Phe Leu Ser Leu Lys His Thr Pro Met Gly Lys Lys 275	280	285
Ser Arg Arg Pro Ser Ala Lys Ser Thr Met Pro Thr Ser Leu Pro Asn 290	295	300
Leu Ala Lys Glu Ala Lys Asp Ala Lys Gly Ser Arg Arg Lys Lys Ser 305	310	315
Leu Ser Glu Lys Val Gln Leu Ser Glu Ser Ser Val Thr Leu Ser Pro 325	330	335
Val Asp Ser Leu Glu Ser Pro His Thr Tyr Val Ser Asp Thr Thr Ser 340	345	350
Ser Pro Met Ile Thr Ser Pro Gly Ile Leu Gln Ala Ser Pro Asn Pro 355	360	365
Met Leu Ala Thr Ala Ala Pro Pro Ala Pro Val His Ala Gln His Ala 370	375	380
Leu Ser Phe Ser Asn Leu His Glu Met Gln Pro Leu Ala His Gly Ala 385	390	395
Ser Thr Val Leu Pro Ser Val Ser Gln Leu Leu Ser His His His Ile 405	410	415
Val Ser Pro Gly Ser Gly Ser Ala Gly Ser Leu Ser Arg Leu His Pro 420	425	430
Val Pro Val Pro Ala Asp Trp Met Asn Arg Met Glu Val Asn Glu Thr 435	440	445
Gln Tyr Asn Glu Met Phe Gly Met Val Leu Ala Pro Ala Glu Gly Thr 450	455	460
His Pro Gly Ile Ala Pro Gln Ser Arg Pro Pro Glu Gly Lys His Ile 465	470	475
Thr Thr Pro Arg Glu Pro Leu Pro Pro Ile Val Thr Phe Gln Leu Ile 485	490	495
Pro Lys Gly Ser Ile Ala Gln Pro Ala Gly Ala Pro Gln Pro Gln Ser 500	505	510
Thr Cys Pro Pro Ala Val Ala Gly Pro Leu Pro Thr Met Tyr Gln Ile 515	520	525
Pro Glu Met Ala Arg Leu Pro Ser Val Ala Phe Pro Thr Ala Met Met 530	535	540
Pro Gln Gln Asp Gly Gln Val Ala Gln Thr Ile Leu Pro Ala Tyr His 545	550	555
Pro Phe Pro Ala Ser Val Gly Lys Tyr Pro Thr Pro Pro Ser Gln His 565	570	575
Ser Tyr Ala Ser Ser Asn Ala Ala Glu Arg Thr Pro Ser His Ser Gly 580	585	590
His Leu Gln Gly Glu His Pro Tyr Leu Thr Pro Ser Pro Glu Ser Pro 595	600	605
Asp Gln Trp Ser Ser Ser Ser Pro His Ser Ala Ser Asp Trp Ser Asp 610	615	620

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Val Thr Thr Ser Pro Thr Pro Gly Gly Ala Gly Gly Gly Gln Arg Gly
 625 630 635 640
 Pro Gly Thr His Met Ser Glu Pro Pro His Asn Asn Met Gln Val Tyr
 645 650 655

Ala

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 654 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Thr Pro Pro Gln Gly Glu Ile Glu Ala Asp Cys Met Asp Val Asn Val
 1 5 10 15
 Arg Gly Pro Asp Gly Phe Thr Pro Leu Met Ile Ala Ser Cys Ser Gly
 20 25 30
 Gly Gly Leu Glu Thr Gly Asn Ser Glu Glu Glu Glu Asp Ala Ser Ala
 35 40 45
 Asn Met Ile Ser Asp Phe Ile Gly Gln Gly Ala Gln Leu His Asn Gln
 50 55 60
 Thr Asp Arg Thr Gly Glu Thr Ala Leu His Leu Ala Ala Arg Tyr Ala
 65 70 75 80
 Arg Ala Asp Ala Ala Lys Arg Leu Leu Glu Ser Ser Ala Asp Ala Asn
 85 90 95
 Val Gln Asp Asn Met Gly Arg Thr Pro Leu His Ala Ala Val Ala Ala
 100 105 110
 Asp Ala Gln Gly Val Phe Gln Ile Leu Ile Arg Asn Arg Ala Thr Asp
 115 120 125
 Leu Asp Ala Arg Met Phe Asp Gly Thr Thr Pro Leu Ile Leu Ala Ala
 130 135 140
 Arg Leu Ala Val Glu Gly Met Val Glu Glu Leu Ile Asn Ala His Ala
 145 150 155 160
 Asp Val Asn Ala Val Asp Glu Phe Gly Lys Ser Ala Leu His Trp Ala
 165 170 175
 Ala Ala Val Asn Asn Val Asp Ala Ala Ala Val Leu Leu Lys Asn Ser
 180 185 190
 Ala Asn Lys Asp Met Gln Asn Asn Lys Glu Glu Thr Ser Leu Phe Leu
 195 200 205
 Ala Ala Arg Glu Gly Ser Tyr Glu Thr Ala Lys Val Leu Leu Asp His
 210 215 220
 Tyr Ala Asn Arg Asp Ile Thr Asp His Met Asp Arg Leu Pro Arg Asp
 225 230 235 240

WHAT IS CLAIMED IS:

1. A substantially purified human Notch protein.
5
2. A substantially purified protein comprising an amino acid sequence encoded by the DNA sequence depicted in Figure 19A (SEQ ID NO:13), 19B (SEQ ID NO:14) or 19C (SEQ ID NO:15).
10
3. A substantially purified protein comprising an amino acid sequence encoded by the DNA sequence depicted in Figure 20A (SEQ ID NO:16), 20B (SEQ ID NO:17), 20C (SEQ ID NO:18), or 20D (SEQ ID
15 NO:19).
4. A substantially purified protein comprising an amino acid sequence encoded by the DNA sequence depicted in Figure 21A (SEQ ID NO:20), or 21B
20 (SEQ ID NO:21).
5. A substantially purified protein comprising an amino acid sequence encoded by the DNA sequence depicted in Figure 22A (SEQ ID NO:22), 22B
25 (SEQ ID NO:23), 22C (SEQ ID NO:24), or 22D (SEQ ID NO:25).
6. A substantially purified protein comprising an amino acid sequence encoded by the DNA
30 sequence depicted in Figure 19A (SEQ ID NO:13), 19B (SEQ ID NO:14), 19C (SEQ ID NO:15), 20A (SEQ ID NO:16), 20B (SEQ ID NO:17), 20C (SEQ ID NO:18), 20D (SEQ ID NO:19), 21A (SEQ ID NO:20), 21B (SEQ ID NO:21), 22A (SEQ ID NO:22), 22B (SEQ ID NO:23), 22C
35

(SEQ ID NO:24), or 22D (SEQ ID NO:25), which is able to be bound by an antibody to a human Notch protein.

7. A substantially purified protein
5 comprising a Notch amino acid sequence encoded by the DNA sequence depicted in Figure 19A (SEQ ID NO:13), 19B (SEQ ID NO:14), 19C (SEQ ID NO:15), 20A (SEQ ID NO:16), 20B (SEQ ID NO:17), 20C (SEQ ID NO:18), 20D (SEQ ID NO:19), 21A (SEQ ID NO:20), 21B (SEQ ID
10 NO:21), 22A (SEQ ID NO:22), 22B (SEQ ID NO:23), 22C (SEQ ID NO:24), or 22D (SEQ ID NO:25) which displays one or more functional activities associated with a full-length Notch protein.

15 8. A substantially purified protein comprising: a fragment of a human Notch protein consisting of at least 77 amino acids.

9. A substantially purified protein
20 comprising: a fragment of a human Notch protein consisting essentially of the extracellular domain of the protein.

10. A substantially purified protein
25 comprising: a fragment of a human Notch protein consisting essentially of the intracellular domain of the protein.

11. A substantially purified protein
30 comprising: a fragment of a human Notch protein consisting essentially of the extracellular and transmembrane domains of the protein.

12. A substantially purified protein
35 comprising: a fragment of a human Notch protein

consisting essentially of the intracellular domain of the protein, as encoded by a portion of plasmid hN3k as deposited with the ATCC and assigned accession number 68609, or as encoded by a portion of plasmid
5 hN5k as deposited with the ATCC and assigned accession number 68611.

13. A substantially purified protein comprising: a fragment of a human Notch protein
10 consisting essentially of the region containing the cdc10 repeats of the protein.

14. A substantially purified protein comprising: a fragment of a human Notch protein
15 consisting essentially of the region containing the cdc10 repeats, as encoded by a portion of plasmid hN3k as deposited with the ATCC and assigned accession number 68611, or as encoded by a portion of plasmid hN5k as deposited with the ATCC and assigned accession
20 number 68611.

15. A substantially purified protein comprising a region of a human Notch protein containing the EGF homologous repeats of the protein.
25

16. A substantially purified protein comprising a region of a human Notch protein containing the Notch/lin-12 repeats of the protein.

30 17. A substantially purified fragment of a human Notch protein substantially lacking the EGF-homologous repeats of the protein, which fragment is able to be bound by an antibody to a Notch protein.

35

18. A substantially purified fragment of a human Notch protein lacking a portion of the EGF-homologous repeats of the protein, which fragment is able to be bound by an antibody to a Notch protein.

5

19. A substantially purified protein comprising an amino acid sequence encoded by at least 121 nucleotides of the human cDNA sequence contained in plasmid hN3k as deposited with the ATCC and
10 assigned accession number 68609.

20. A substantially purified protein comprising an amino acid sequence encoded by at least 121 nucleotides of the human cDNA sequence contained
15 in plasmid hN4k as deposited with the ATCC and assigned accession number 68610.

21. A substantially purified protein comprising an amino acid sequence encoded by at least
20 121 nucleotides of the human cDNA sequence contained in plasmid hN5k as deposited with the ATCC and assigned accession number 68611.

22. A substantially purified fragment of a
25 human Notch protein consisting essentially of the intracellular domain of the protein.

23. A substantially purified fragment of a human Notch protein consisting essentially of the
30 extracellular domain of the protein.

24. A substantially purified fragment of a human Notch protein consisting essentially of the extracellular and transmembrane domains of the
35 protein.

25. A chimeric protein comprising the fragment of claim 8 joined to a heterologous protein sequence.

5 26. A chimeric protein comprising the fragment of claim 9 joined to a heterologous protein sequence.

10 27. A substantially purified protein comprising a functionally active portion of a human Notch protein.

15 28. A substantially purified protein comprising a functionally active portion of the Notch protein sequence encoded by the human cDNA sequence contained in plasmid hN3k as deposited with the ATCC and assigned accession number 68609, or encoded by the human cDNA sequence contained in plasmid hN5k as deposited with the ATCC and assigned accession number
20 68611.

25 29. A substantially purified protein comprising a functionally active portion of the Notch protein sequence encoded by the human cDNA sequence contained in plasmid hN4k as deposited with the ATCC and assigned accession number 68610.

30 30. A substantially purified protein comprising the amino acid sequence depicted in Figure 23.

35 31. A substantially purified protein comprising the amino acid sequence depicted in Figure 24.

32. A substantially purified protein comprising the Notch amino acid sequence encoded by the human Notch DNA sequence contained in plasmid hN3k as deposited with the ATCC and assigned accession
5 number 68609.

33. A substantially purified protein comprising the Notch amino acid sequence encoded by the human Notch DNA sequence contained in plasmid hN5k
10 as deposited with the ATCC and assigned accession number 68611.

34. A fragment of the protein of claim 30 which is characterized by the ability in vitro, when
15 expressed on the surface of a first cell, to bind to a Delta protein expressed on the surface of a second cell.

35. A fragment of the protein of claim 31
20 which is characterized by the ability in vitro, when expressed on the surface of a first cell, to bind to a Delta protein expressed on the surface of a second cell.

25 36. A substantially purified protein comprising the portion of a human Notch protein with the greatest homology to the epidermal growth factor-like repeats 11 and 12 of the Drosophila Notch sequence as shown in Figure 8 (SEQ ID NO:1).

30 37. A derivative or analog of the protein of claim 1, which is characterized by the ability in vitro, when expressed on the surface of a first cell, to bind to a Delta protein expressed on the surface of
35 a second cell.

38. A chimeric protein comprising the protein of claim 1 joined to a heterologous protein sequence.

5 39. A chimeric protein comprising the protein of claim 6 joined to a heterologous protein sequence.

10 40. A chimeric protein comprising the protein of claim 7 joined to a heterologous protein sequence.

41. A substantially purified fragment of a Notch protein, which is characterized by the ability
15 in vitro, when expressed on the surface of a first cell, to bind to a Delta protein expressed on the surface of a second cell.

42. The fragment of claim 41 consisting
20 essentially of the portion of the Notch protein with the greatest homology to the epidermal growth factor-like repeats 11 and 12 of the Drosophila Notch protein.

25 43. The fragment of claim 41 in which the Notch protein is a Drosophila Notch protein.

44. The fragment of claim 41 in which the Notch protein is a Xenopus Notch protein.
30

45. The fragment of claim 41 in which the Notch protein is a human Notch protein.

35

46. A chimeric protein comprising the fragment of claim 45 joined to a heterologous protein sequence.

5 47. A substantially purified fragment of a Drosophila Notch protein consisting essentially of the epidermal growth factor-like repeats 11 and 12 of the protein.

10 48. A chimeric protein comprising the fragment of claim 41 or 47 joined to a heterologous protein sequence.

15 49. A substantially purified fragment of a Delta protein, which is characterized by the ability in vitro, when expressed on the surface of a first cell, to bind to a Notch protein expressed on the surface of a second cell.

20 50. The fragment of claim 49 which is the portion of the Delta protein with the greatest homology to amino acid numbers 1-230 as depicted in Figure 13 (SEQ ID NO:6).

25 51. A chimeric protein comprising the fragment of claim 49 joined to a heterologous protein sequence.

30 52. A substantially purified fragment of a Delta protein, which is characterized by the ability in vitro, when expressed on the surface of a first cell, to bind to a second Delta protein or fragment expressed on the surface of a second cell.

35

53. The fragment of claim 52 which is the portion of the Delta protein with the greatest homology to about amino acid numbers 32-230 as depicted in Figure 13 (SEQ ID NO:6).

5

54. A chimeric protein comprising the fragment of claim 52 joined to a heterologous protein sequence.

10

55. A substantially purified fragment of a Serrate protein, which is characterized by the ability in vitro, when expressed on the surface of a first cell, to bind to a Notch protein expressed on the surface of a second cell.

15

56. A substantially purified fragment of a Serrate protein which is the portion of the Serrate protein with the greatest homology to the amino acid sequence as depicted in Figure 15 (SEQ ID NO:9) from about amino acid numbers 85-283.

20

57. A chimeric protein comprising the fragment of claim 56 joined to a heterologous protein sequence.

25

58. A derivative or analog of the fragment of claim 41 which is characterized by the ability in vitro, when expressed on the surface of a first cell, to bind to a Delta protein expressed on the surface of a second cell.

30

59. A derivative or analog of the fragment of claim 49, which is characterized by the ability in vitro, when expressed on the surface of a first cell,

35

to bind to a Notch protein expressed on the surface of a second cell.

60. A derivative or analog of the fragment
5 of claim 52, which is characterized by the ability in vitro, when expressed on the surface of a first cell, to bind to a second Delta protein expressed on the surface of a second cell.

10 61. A derivative or analog of the fragment of claim 55, which is characterized by the ability in vitro, when expressed on the surface of a first cell, to bind to a second protein expressed on the surface of a second cell, which second protein is selected
15 from the group consisting of a Notch protein, a Delta protein, and a second Serrate protein.

62. A substantially purified fragment of a
human Notch protein consisting of at least 40 amino
20 acids.

63. A substantially purified nucleic acid encoding a human Notch protein.

25 64. A substantially purified nucleic acid comprising a cDNA sequence encoding a human Notch protein.

65. A substantially purified nucleic acid
30 comprising a nucleotide sequence complementary to and capable of hybridizing to the cDNA sequence of claim 64.

35

66. A substantially purified cDNA sequence encoding a functionally active portion of a human Notch protein.

5 67. A substantially purified nucleic acid comprising a nucleotide sequence complementary to and capable of hybridizing to the cDNA sequence of claim 66.

10 68. A substantially purified cDNA molecule comprising the DNA sequence depicted in Figure 19A (SEQ ID NO:13), 19B (SEQ ID NO:14), 19C (SEQ ID NO:15), 20A (SEQ ID NO:16), 20B (SEQ ID NO:17), 20C (SEQ ID NO:18), 20D (SEQ ID NO:19), 21A (SEQ ID
15 NO:20), 21B (SEQ ID NO:21), 22A (SEQ ID NO:22), 22B (SEQ ID NO:23), 22C (SEQ ID NO:24), or 22D (SEQ ID NO:25).

 69. The nucleic acid of claim 63 in which
20 the Notch protein comprises an amino acid sequence encoded by the DNA sequence depicted in Figure 19A (SEQ ID NO:13), 19B (SEQ ID NO:14), 19C (SEQ ID NO:15), 20A (SEQ ID NO:16), 20B (SEQ ID NO:17), 20C (SEQ ID NO:18), 20D (SEQ ID NO:19), 21A (SEQ ID
25 NO:20), 21B (SEQ ID NO:21), 22A (SEQ ID NO:22), 22B (SEQ ID NO:23), 22C (SEQ ID NO:24), or 22D (SEQ ID NO:25).

 70. A substantially purified nucleic acid
30 comprising a DNA sequence encoding at least a 77 amino acid portion of a human Notch protein, which portion has the greatest homology to the epidermal growth factor-like repeats 11 and 12 of the Drosophila Notch sequence as shown in Figure 8 (SEQ ID NO:1).

35

71. A substantially purified nucleic acid comprising the human Notch cDNA contained in plasmid hN4k, as deposited with the ATCC and assigned accession number 68610.

5

72. A substantially purified nucleic acid comprising the human Notch cDNA contained in plasmid hN3k, as deposited with the ATCC and assigned accession number 68609.

10

73. A substantially purified nucleic acid comprising the human Notch cDNA contained in plasmid hN5k, as deposited with the ATCC and assigned accession number 68611.

15

74. A substantially purified nucleic acid comprising the DNA coding sequence depicted in Figure 23.

20

75. A substantially purified nucleic acid comprising the DNA coding sequence depicted in Figure 24.

76. A substantially purified nucleic acid comprising a cDNA sequence encoding the extracellular domain of a human Notch protein.

77. A substantially purified nucleic acid comprising a cDNA sequence encoding the intracellular domain of a human Notch protein.

78. A substantially purified nucleic acid comprising a cDNA sequence encoding the extracellular and transmembrane domains of a human Notch protein.

35

79. A substantially purified nucleic acid comprising a cDNA sequence encoding the EGF-homologous repeats of a human Notch protein.

5 80. A substantially purified nucleic acid comprising a cDNA sequence encoding the Notch/lin-12 repeats of a human Notch protein.

10 81. A substantially purified cDNA molecule encoding a fragment of a human Notch protein of at least 77 amino acids.

15 82. A substantially purified cDNA molecule encoding a fragment of a human Notch protein of at least 40 amino acids.

20 83. A substantially purified nucleic acid encoding the amino acid sequence depicted in Figure 23.

 84. A substantially purified nucleic acid encoding the amino acid sequence depicted in Figure 24.

25 85. A substantially purified nucleic acid encoding the protein of claim 36.

30 86. A substantially purified nucleic acid encoding the fragment of claim 41.

 87. A substantially purified nucleic acid encoding the fragment of claim 45.

35 88. A substantially purified nucleic acid encoding the fragment of claim 47.

89. A substantially purified nucleic acid encoding the fragment of claim 49.

90. A substantially purified nucleic acid
5 encoding the fragment of claim 52.

91. A substantially purified nucleic acid encoding the fragment of claim 55.

10 92. A nucleic acid encoding the chimeric protein of claim 48.

93. A nucleic acid encoding the chimeric protein of claim 51.

15 94. A nucleic acid encoding the chimeric protein of claim 54.

95. A nucleic acid vector comprising the
20 nucleic acid of claim 63.

96. A nucleic acid vector comprising the cDNA molecule of claim 66.

25 97. A nucleic acid vector comprising the nucleic acid of claim 85.

98. A nucleic acid vector comprising the nucleic acid of claim 86.

30 99. A nucleic acid vector comprising the nucleic acid of claim 87.

100. A nucleic acid vector comprising the
35 nucleic acid of claim 88.

101. A nucleic acid vector comprising the nucleic acid of claim 89.

5 102. A nucleic acid vector comprising the nucleic acid of claim 91.

103. A recombinant cell containing the nucleic acid vector of claim 95.

10

104. A recombinant cell containing the nucleic acid vector of claim 96.

15 105. A recombinant cell containing the nucleic acid vector of claim 97.

106. A recombinant cell containing the nucleic acid vector of claim 98.

20 107. A recombinant cell containing the nucleic acid vector of claim 99.

108. A recombinant cell containing the nucleic acid vector of claim 100.

25

109. A recombinant cell containing the nucleic acid vector of claim 101.

30 110. A recombinant cell containing the nucleic acid vector of claim 102.

111. A method for producing a human Notch protein comprising growing the recombinant cell of claim 103, such that the human Notch protein is

35

expressed by the cell; and isolating the expressed human Notch protein.

112. A method for producing a portion of a human Notch protein comprising growing the recombinant cell of claim 104, such that the portion of human Notch is expressed by the cell; and isolating the expressed human Notch portion.

113. A method for producing a protein comprising growing the recombinant cell of claim 105 such that the protein is expressed by the cell; and isolating the expressed protein.

114. A method for producing a fragment of a Notch protein comprising growing the recombinant cell of claim 106 such that the fragment is expressed by the cell; and isolating the expressed fragment of a Notch protein.

115. A method for producing a fragment of a human Notch protein comprising growing the recombinant cell of claim 107 such that the fragment is expressed by the cell; and isolating the expressed fragment of a human Notch protein.

116. A method for producing a fragment of a Drosophila Notch protein comprising growing the recombinant cell of claim 108 such that the fragment is expressed by the cell; and isolating the expressed fragment of a Drosophila Notch protein.

117. A method for producing a fragment of a Delta protein comprising growing the recombinant cell of claim 109 such that the fragment is expressed by

the cell; and isolating the expressed fragment of a Delta protein.

118. A method for producing a fragment of a
5 Serrate protein comprising growing the recombinant cell of claim 110 such that the fragment is expressed by the cell; and isolating the expressed fragment of a Serrate protein.

10 119. An antibody which binds to a human Notch protein and which does not bind to a Drosophila Notch protein.

120. An antibody which binds to the
15 fragment of claim 41.

121. An antibody which binds to the fragment of claim 49.

20 122. An antibody which binds to the fragment of claim 52.

123. An antibody which binds to the fragment of claim 55.

25

124. A fragment or derivative of the antibody of claim 119 containing the idiotype of the antibody.

30 125. A fragment or derivative of the antibody of claim 120 containing the idiotype of the antibody.

126. An antibody which binds to the Notch
35 protein sequence encoded by plasmid hN3k, as deposited

with the ATCC and assigned accession number 68609, or to the Notch protein sequence encoded by plasmid hN5k, as deposited with the ATCC and assigned accession number 68611, and which does not bind to a Drosophila Notch protein.

127. A substantially purified nucleic acid which encodes a protein or peptide which comprises (a) a first amino acid sequence homologous to both a Serrate protein and a Delta protein; and (b) a second amino acid sequence which is not homologous to either a Serrate protein or a Delta protein.

128. A substantially purified fragment of a Notch protein, which is characterized by the ability in vitro, when expressed on the surface of a first cell, to bind to a Serrate protein expressed on the surface of a second cell.

129. A substantially purified fragment of a Serrate protein which is the portion of the Serrate protein with the greatest homology to the amino acid sequence as depicted in Figure 15 (SEQ ID NO:9) from about amino acid numbers 79-282.

130. A substantially purified fragment or derivative of a Delta protein, which is characterized by (a) the ability in vitro, when expressed on the surface of a first cell to bind to a second Delta protein or fragment or derivative expressed on the surface of a second cell; and (b) the inability, in vitro, when expressed on the surface of a third cell, to bind to a Notch protein expressed on the surface of a fourth cell.

131. A method of delivering an agent into a cell expressing a Notch protein comprising exposing a Notch-expressing cell to a molecule such that the molecule is delivered into the cell, in which the

5 molecule comprises a Delta protein or Delta fragment or derivative bound to an agent, in which the Delta protein, fragment, or derivative is characterized by the ability, in vitro, when expressed on the surface of a first cell, to bind to a Notch protein expressed

10 on the surface of a second cell.

132. An isolated nucleic acid comprising at least 25 nucleotides of the DNA coding sequence depicted in Figure 23 or 24.

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G GAG GTG GAT GTG TTA GAT GTG AAT GTC CGT GGC CCA GAT GGC TGC	46
Glu Val Asp Val Leu Asp Val Asn Val Arg Gly Pro Asp Gly Cys	
1 5 10 15	
ACC CCA TTG ATG TTG GCT TCT CTC CGA GGA GGC AGC TCA GAT TTG AGT	94
Thr Pro Leu Met Leu Ala Ser Leu Arg Gly Gly Ser Ser Asp Leu Ser	
20 25 30	
GAT GAA GAT GAA GAT GCA GAG GAC TCT TCT GCT AAC ATC ATC ACA GAC	142
Asp Glu Asp Glu Asp Ala Glu Asp Ser Ser Ala Asn Ile Ile Thr Asp	
35 40 45	
TTG GTC TAC CAG GGT GCC AGC CTC CAG GCC CAG ACA GAC CGG ACT GGT	190
Leu Val Tyr Gln Gly Ala Ser Leu Gln Ala Gln Thr Asp Arg Thr Gly	
50 55 60	
GAG ATG GCC CTG CAC CTT GCA GCC CGC TAC TCA CGG GCT GAT GCT GCC	238
Glu Met Ala Leu His Leu Ala Ala Arg Tyr Ser Arg Ala Asp Ala Ala	
65 70 75	
AAG CGT CTC CTG GAT GCA GGT GCA GAT GCC AAT GCC CAG GAC AAC ATG	286
Lys Arg Leu Leu Asp Ala Gly Ala Asp Ala Asn Ala Gln Asp Asn Met	
80 85 90 95	
GGC CGC TGT CCA CTC CAT GCT GCA GTG GCA GCT GAT GCC CAA GGT GTC	334
Gly Arg Cys Pro Leu His Ala Ala Val Ala Ala Asp Ala Gln Gly Val	
100 105 110	
TTC CAG ATT CTG ATT CGC AAC CGA GTA ACT GAT CTA GAT GCC AGG ATG	382
Phe Gln Ile Leu Ile Arg Asn Arg Val Thr Asp Leu Asp Ala Arg Met	
115 120 125	
AAT GAT GGT ACT ACA CCC CTG ATC CTG GCT GCC CGC CTG GCT GTG GAG	430
Asn Asp Gly Thr Thr Pro Leu Ile Leu Ala Ala Arg Leu Ala Val Glu	
130 135 140	

FIG.24A

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GGA ATG GTG GCA GAA CTG ATC AAC TGC CAA GCG GAT GTG AAT GCA GTG	478
Gly Met Val Ala Glu Leu Ile Asn Cys Gln Ala Asp Val Asn Ala Val	
145 150 155	
GAT GAC CAT GGA AAA TCT GCT CTT CAC TGG GCA GCT GCT GTC AAT AAT	526
Asp Asp His Gly Lys Ser Ala Leu His Trp Ala Ala Ala Val Asn Asn	
160 165 170 175	
GTG GAG GCA ACT CTT TTG TTG TTG AAA AAT GGG GCC AAC CGA GAC ATG	574
Val Glu Ala Thr Leu Leu Leu Lys Asn Gly Ala Asn Arg Asp Met	
180 185 190	
CAG GAC AAC AAG GAA GAG ACA CCT CTG TTT CTT GCT GCC CGG GAG GGG	622
Gln Asp Asn Lys Glu Glu Thr Pro Leu Phe Leu Ala Ala Arg Glu Gly	
195 200 205	
AGC TAT GAA GCA GCC AAG ATC CTG TTA GAC CAT TTT GCC AAT CGA GAC	670
Ser Tyr Glu Ala Ala Lys Ile Leu Leu Asp His Phe Ala Asn Arg Asp	
210 215 220	
ATC ACA GAC CAT ATC GAT CGT CTT CCC CGG GAT GTG GCT CGG GAT CGC	718
Ile Thr Asp His Met Asp Arg Leu Pro Arg Asp Val Ala Arg Asp Arg	
225 230 235	
ATG CAC CAT GAC ATT GTG CGC CTT CTG GAT GAA TAC AAT GTG ACC CCA	766
Met His His Asp Ile Val Arg Leu Leu Asp Glu Tyr Asn Val Thr Pro	
240 245 250 255	
AGC CCT CCA GGC ACC GTG TTG ACT TCT GCT CTC TCA CCT GTC ATC TGT	814
Ser Pro Pro Gly Thr Val Leu Thr Ser Ala Leu Ser Pro Val Ile Cys	
260 265 270	
GGG CCC AAC AGA TCT TTC CTC AGC CTG AAG CAC ACC CCA ATG GGC AAG	862
Gly Pro Asn Arg Ser Phe Leu Ser Leu Lys His Thr Pro Met Gly Lys	
275 280 285	

FIG.24B

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AAG TCT AGA CGG CCC AGT GCC AAG AGT ACC ATG CCT ACT AGC CTC CCT	910
Lys Ser Arg Arg Pro Ser Ala Lys Ser Thr Met Pro Thr Ser Leu Pro	
290 295 300	
AAC CTT GCC AAG GAG GCA AAG GAT GCC AAG GGT AGT AGG AGG AAG AAG	958
Asn Leu Ala Lys Glu Ala Lys Asp Ala Lys Gly Ser Arg Arg Lys Lys	
305 310 315	
TCT CTG AGT GAG AAG GTC CAA CTG TCT GAG AGT TCA GTA ACT TTA TCC	1006
Ser Leu Ser Glu Lys Val Gln Leu Ser Glu Ser Ser Val Thr Leu Ser	
320 325 330 335	
CCT GTT GAT TCC CTA GAA TCT CCT CAC ACG TAT GTT TCC GAC ACC ACA	1054
Pro Val Asp Ser Leu Glu Ser Pro His Thr Tyr Val Ser Asp Thr Thr	
340 345 350	
TCC TCT CCA ATG ATT ACA TCC CCT GGG ATC TTA CAG GCC TCA CCC AAC	1102
Ser Ser Pro Met Ile Thr Ser Pro Gly Ile Leu Gln Ala Ser Pro Asn	
355 360 365	
CCT ATG TTG GCC ACT GCC GCC CCT CCT GCC CCA GTC CAT GCC CAG CAT	1150
Pro Met Leu Ala Thr Ala Ala Pro Pro Ala Pro Val His Ala Gln His	
370 375 380	
GCA CTA TCT TTT TCT AAC CTT CAT GAA ATG CAG CCT TTG GCA CAT GGG	1198
Ala Leu Ser Phe Ser Asn Leu His Glu Met Gln Pro Leu Ala His Gly	
385 390 395	
GCC AGC ACT GTG CTT CCC TCA GTG AGC CAG TTG CTA TCC CAC CAC CAC	1246
Ala Ser Thr Val Leu Pro Ser Val Ser Gln Leu Leu Ser His His His	
400 405 410 415	
ATT GTG TCT CCA GGC AGT GGC AGT GCT GGA AGC TTG AGT AGG CTC CAT	1294
Ile Val Ser Pro Gly Ser Gly Ser Ala Gly Ser Leu Ser Arg Leu His	
420 425 430	
CCA GTC CCA GTC CCA GCA GAT TGG ATG AAC CGC ATG GAG GTG AAT GAG	1342
Pro Val Pro Val Pro Ala Asp Trp Met Asn Arg Met Glu Val Asn Glu	
435 440 445	

FIG.24C
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ACC CAG TAC AAT GAG ATG TTT GGT ATG GTC CTG GCT CCA GCT GAG GGC 1390
 Thr Gln Tyr Asn Glu Met Phe Gly Met Val Leu Ala Pro Ala Glu Gly
 450 455 460

ACC CAT CCT GGC ATA GCT CCC CAG AGC AGG CCA CCT GAA GGG AAG CAC 1438
 Thr His Pro Gly Ile Ala Pro Gln Ser Arg Pro Pro Glu Gly Lys His
 465 470 475

ATA ACC ACC CCT CGG GAG CCC TTG CCC CCC ATT GTG ACT TTC CAG CTC 1486
 Ile Thr Thr Pro Arg Glu Pro Leu Pro Pro Ile Val Thr Phe Gln Leu
 480 485 490 495

ATC CCT AAA GGC AGT ATT GCC CAA CCA GCG GGG GCT CCC CAG CCT CAG 1534
 Ile Pro Lys Gly Ser Ile Ala Gln Pro Ala Gly Ala Pro Gln Pro Gln
 500 505 510

TCC ACC TGC CCT CCA GCT GTT GCG GGC CCC CTG CCC ACC ATG TAC CAG 1582
 Ser Thr Cys Pro Pro Ala Val Ala Gly Pro Leu Pro Thr Met Tyr Gln
 515 520 525

ATT CCA GAA ATG GCC CGT TTG CCC AGT GTG GCT TTC CCC ACT GCC ATG 1630
 Ile Pro Glu Met Ala Arg Leu Pro Ser Val Ala Phe Pro Thr Ala Met
 530 535 540

ATG CCC CAG CAG GAC GGG CAG GTA GCT CAG ACC ATT CTC CCA GCC TAT 1678
 Met Pro Gln Gln Asp Gly Gln Val Ala Gln Thr Ile Leu Pro Ala Tyr
 545 550 555

CAT CCT TTC CCA GCC TCT GTG GGC AAG TAC CCC ACA CCC CCT TCA CAG 1726
 His Pro Phe Pro Ala Ser Val Gly Lys Tyr Pro Thr Pro Pro Ser Gln
 560 565 570 575

CAC AGT TAT GCT TCC TCA AAT GCT GCT GAG CGA ACA CCC AGT CAC AGT 1774
 His Ser Tyr Ala Ser Ser Asn Ala Ala Glu Arg Thr Pro Ser His Ser
 580 585 590

GGT CAC CTC CAG GGT GAG CAT CCC TAC CTG ACA CCA TCC CCA GAG TCT 1822
 Gly His Leu Gln Gly Glu His Pro Tyr Leu Thr Pro Ser Pro Glu Ser
 595 600 605

FIG.24D

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CCT GAC CAG TGG TCA AGT TCA TCA CCC CAC TCT GCT TCT GAC TGG TCA	1870
Pro Asp Gln Trp Ser Ser Ser Ser Pro His Ser Ala Ser Asp Trp Ser	
610 615 620	
GAT GTG ACC ACC AGC CCT ACC CCT GGG GGT GCT GGA GGA GGT CAG CGG	1918
Asp Val Thr Thr Ser Pro Thr Pro Gly Gly Ala Gly Gly Gly Gln Arg	
625 630 635	
GGA CCT GGG ACA CAC ATG TCT GAG CCA CCA CAC AAC AAC ATG CAG GTT	1966
Gly Pro Gly Thr His Met Ser Glu Pro Pro His Asn Asn Met Gln Val	
640 645 650 655	
TAT GCG TGAGAGAGTC CACCTCCAGT GTAGAGACAT AACTGACTTT TGTAATGCT	2022
Tyr Ala	
GCTGAGGAAC AAATGAAGGT CATCCGGGAG AGAAATGAAG AAATCTCTGG AGCCAGCTTC	2082
TAGAGGTAGG AAAGAGAAGA TGTTCATTATT CAGATAATGC AAGAGAAGCA ATTCGTCAGT	2142
TTCACCTGGGT ATCTGCAAGG CTTATTGATT ATTCTAATCT AATAAGACAA GTTTGTGGAA	2202
ATGCAAGATG AATACAAGCC TTGGGTCCAT GTTTACTCTC TTCTATTTGG AGAATAAGAT	2262
GGATGCTTAT TGAAGCCCAG ACATTCTTGC AGCTTGGACT GCATTTTAAG CCCTGCAGGC	2322
TTCTGCCATA TCCATGAGAA GATTCTACAC TAGCGTCTG TTGGGAATTA TGCCCTGGAA	2382
TTCTGCCTGA ATTGACCTAC GCATCTCCTC CTCCTTGGAC ATTCTTTTGT CTTCAATTTGG	2442
TGCTTTTGGT TTTGCACCTC TCCGTGATTG TAGCCCTACC AGCATGTTAT AGGGCAAGAC	2502
CTTTGTGCTT TTGATCATTG TGGCCCATGA AAGCAACTTT GGTCTCCTTT CCCCTCCTGT	2562
CTTCCCGGTA TCCCTTGGAG TCTCACAAGG TTTACTTTGG TATGGTTCTC AGCACAACC	2622
TTTCAAGTAT GTTGTTCCTT TGGAAAATGG ACATACTGTA TTGTGTTCTC CTGCATATAT	2682
CATTCCTGGA GAGAGAAGGG GAGAAGAATA CTTTCTTCA ACAAATTTTG GGGGCAGGAG	2742
ATCCCTTCAA GAGGCTGCAC CTTAATTTTT CTTGTCTGTG TGCAGGTCTT CATATAAAT	2802

FIG.24E

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TTACCAGGAA GAAGGGTGTG AGTTTGTGT TTTTCTGTGT ATGGGCCTGG TCAGTGTA	2862
TTTTATCCT TGATAGTCTA GTTACTATGA CCCTCCCCAC TTTTAAAA CCAGAAAAAG	2922
TTTTGGAATG TTGGAATGAC CAAGAGACAA GTTAACTCGT GCAAGAGCCA GTTACCCACC	2982
CACAGGTCCC CCTACTTCCT GCCAAGCATT CCATTGACTG CCTGTATGGA ACACATTGT	3042
CCCAGATCTG AGCATTCTAG GCCTGTTTCA CTCCTCACC CAGCATATGA AACTAGTCTT	3102
AACTGTTGAG CCTTTCCTTT CATATCCACA GAAGACACTG TCTCAAATGT TGTACCCCTG	3162
CCATTTAGGA CTGAACCTTC CTTAGCCCAA GGGACCCAGT GACAGTTGTC TTCCGTTTGT	3222
CAGATGATCA GTCTCTACTG ATTATCTTGC TGCTTAAAGG CCTGCTCACC AATCTTTCTT	3282
TCACACCGTG TGGTCCGTGT TACTGGTATA CCCAGTATGT TCTCACTGAA GACATGGACT	3342
TTATATGTTT AAGTGCAGGA ATTGGAAAGT TGGACTTGTT TTCTATGATC CAAAACAGCC	3402
CTATAAGAAG GTTGGAAAAG GAGGAACTAT ATAGCAGCCT TTGCTATTTT CTGCTACCAT	3462
TTCTTTTCCT CTGAAGCGGC CATGACATTC CCTTTGGCAA CTAACGTAGA AACTCAACAG	3522

FIG.24F

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AACATTTTCC TTTCCTAGAG TCACCTTTTA GATGATAATG GACAACTATA GACTTGCTCA	3582
TTGTTTCAGAC TGATTGCCCC TCACCTGAAT CCACTCTCTG TATTCATGCT CTGGGCAATT	3642
TCTTTGACTT TCTTTTAAGG GCAGAAGCAT TTTAGTTAAT TGTAAGATAA GAATAGTTTT	3702
CTTCCTCTTC TCCTTGGGCC AGTTAATAAT TGGTCCATGG CTACACTGCA ACTTCCGTCC	3762
AGTGCTGTGA TGCCCATGAC ACCTGCAAAA TAAGTTCTGC CTGGGCATTT TGTAAGATATT	3822
AACAGGTGAA TTCCCGACTC TTTTGGTTTG AATGACAGTT CTCATTCCCT CTATGGCTGC	3882
AAGTATGCAT CAGTGCTTCC CACTTACCTG ATTTGTCTGT CGGTGGCCCC ATATGGAAAC	3942
CCTGCGTGTC TGTTGGCATA ATAGTTTACA AATGGTTTTT TCAGTCCTAT CCAAATTTAT	4002
TGAACCAACA AAAATAATTA CTCTGCCCT GAGATAAGCA GATTAAGTT GTTCATTCTC	4062
TGCTTTATTC TCTCCATGTG GCAACATTCT GTCAGCCTCT TTCATAGTGT GCAAACATTT	4122
TATCATTCTA AATGGTGACT CTCTGCCCTT GGACCCATTT ATTATTCACA GATGGGGAGA	4182
ACCTATCTGC ATGGACCCCTC ACCATCCTCT GTGCAGCACA CACAGTGCAG GGAGCCAGTG	4242
GCGATGGCGA TGACTTTCTT CCCCTG	4268

FIG.24G

SUBSTITUTE SHEET



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